



Isolation and Molecular Profiling Of Thermoresistant Bacteria From Chimneys Of Baking Industries Of Madurai, Tamilnadu, India

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Abstract: The present study was chosen to discover the thermophiles isolation from chimneys of baking industries of Madurai, Tamilnadu, India. An attempt has been made to unearth the conserved sequences among them. Phylogenetic neighbour joining tree based on their 16S rRNA showing a relationship with related organisms has been constructed for each isolates keeping a species as an outgroup. In Madurai, baking industries have been found to be one of the hottest area having temperature as high as 120°C. Incubations of the bacterial colonies were done with covered water bath incubator at 70°C for 72-96 hrs in Castenholz TYE broth. The temperature range of 40-100°C, pH range of 4.0-10.0 and incubation period of 24-144 hrs was investigated for the best growth of thermophilic bacteria. Samples were collected in sterilized screw capped vials from different sites of Madurai Baking Industries and brought to the laboratory and kept at 4°C in the refrigerator till further processing. PCR products were sequenced using same primers by a commercial sequencing facility (Xcleris lab). The sequences of these ten bacterial isolates after sequencing were blasted using online NCBI BLAST program. Ten thermotolerant bacterial isolates were isolated and the optimal temperature for growth of the isolates was 70°C and the optimal pH was 7.0. 16S rRNA gene sequences of all these thermophilic bacterial isolates exhibited 98-99% homology with *Bacillus* sp. The phylogenetic analysis of the 16S rRNA gene sequences also confirmed the relationship of these thermophilic isolates with the genus *Bacillus*. The 16S rRNA gene of all isolates was amplified, sequenced, and *insilico* analysis of these ten sequences was carried out. Based on BLAST alignment of 16S rRNA gene sequences of these isolates to GenBank sequences, these all isolates belonged to genus *Bacillus* and showed 98-99% similarity. The present study delves in to isolation, identification, characterisation, and analysis of the genomes of bacterial isolates such as *Bacillus* sp. VP1, *Bacillus* sp. VP2, *Bacillus* sp. VP3, *Pseudomonas* sp. VP4, *Pseudomonas* sp. VP5, *Bacillus licheniformis* strain VP7, *Bacillus* sp. VP8, *Bacillus* sp. VP9, *Bacillus altitudinis* sp. VP10, and *Bacillus cereus* strain VP11.

Keywords: Chimneys, Thermoresistant, 16S rRNA, *Bacillus*, Phylogenetic analysis.

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I. INTRODUCTION

A thermophile is an organism that thrives in relatively high temperatures, between 45°C to 80°C. The enzymes of thermophiles can function even at high temperature and they are used extensively in molecular biology (for example, heat-stable DNA polymerases for PCR), and also as washing agents. Advances in molecular biology, bioinformatics and cultivation technologies herald a new age of exploration of microbial world. Since the discovery of *Thermusaquaticus* at Yellowstone National park, newer and different habitats are being explored to understand microbial diversity over time. A great bacterial diversity has been reported in various solar heated environments and phylogenetic research also showed that thermophiles are abundant in many more extreme environments^{1,2}. In India, thermal springs are scattered throughout the country. Thermophilic strains have also been recovered from various thermal springs in India^{3,4}. In Madurai, baking industries has been found to be one of the hottest area having temperature as high as 120°C. The present study was undertaken to discover the thermophiles isolated from chimneys of baking industries of Madurai, Tamilnadu, India.

2. MATERIALS AND METHODS

Samples were collected in a sterilized screw capped vials from different sites of Madurai Baking Industries and brought to the laboratory and kept at 4°C in the refrigerator till further processing. Culture media used for the isolation of thermophilic bacteria were Castenholz TYE medium⁵. All the incubations were done in a covered water bath incubator at 70°C for 3-4 days in Castenholz TYE broth. The temperature range of 40-100°C, pH range of 4.0-10.0 and incubation period of 24-144 hrs was investigated for the best growth of thermophilic bacteria. Thermophilic bacterial isolates have also been studied for various morphological and biochemical characters^{6,7}. For molecular characterization, DNA was extracted from each thermophilic bacterial isolate using Genomic DNA extraction Mini-Kit (Real Genomics). All fragments were amplified from genomic DNA¹⁴. DNA was mixed with 6-fold concentrated low molecular weight glycerol in the loading buffer and separated in 0.8% Agarose Gels prepared in TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8). 1kb DNA ladder (GibcoBRE) was used as a DNA size marker for linear DNA fragments, the agarose gel electrophoresis was performed on the EBF buffer at 100V (Gel Electrophoresis Apparatus GNA 100; Pharmacia Biotech). The agarose gels were stained with a 5µg/ml ethidium bromide (Sigma) solution for 20 min and incubated in distilled water for 20 min to remove the surplus of ethidium bromide from the gels. The DNA in the agarose gels was visualized using ultraviolet light at a wavelength of 302 nm and subsequently photographed (Alpha imager TM 2000, Alpha Innotech). PCR amplification of the 16S rRNA gene was carried out by using the forward primer (5'-GGTCAGCGCGGACGGGTGAGTAAC-3') and the reverse primer (5'-GACGGGCGGTGTGACAGAGGCCCG-3'). PCR products were sequenced using same primers by a commercial sequencing facility (Xcleris lab).

2.1 Phylogenetic analysis (clustal w)

The sequences of bacterial isolates after sequencing were blasted using online NCBI BLAST program⁸. Phylogenetic analysis began with aligning of sequences using tools like Clustal W and after alignment, phylogenetic tree was constructed using MEGA 6.0 software. CLUSTAL W is a tool written in C and C++ and makes use of a number of excellent free software packages. In this work, we used a modified version of Sean Eddy's Squid library for the sequence I/O, allowing the use of a wide variety of file formats and then David Arthur's k-means++ code (Arthur and Vassilvitskii, 2007) for fast clustering of sequence vectors. Code for fast UPGMA and guide tree, handling routines adopted from MUSCLE (Edgar, 2004). We use the OpenMP library to enable multithreaded computation of pairwise distances and alignment match states. CLUSTAL_W is licensed under the GNU Lesser General Public License. Source code, as well as precompiled binaries for Windows, Linux, FreeBSD and Mac (Intel and PowerPC), are available at <http://www.clustal.org> as a command-line program only, which uses GNU-style command-line options, and also accepts CLUSTAL_W-style command options for backward compatibility and easy integration into existing pipelines.

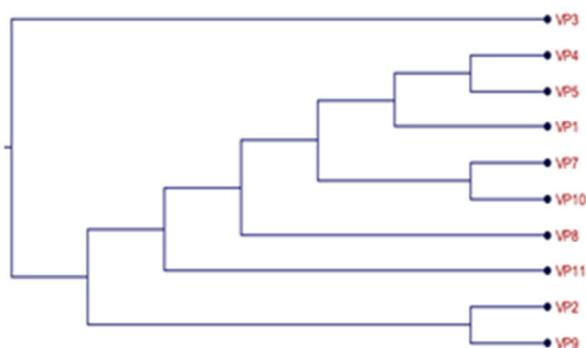
3. RESULTS AND DISCUSSION

In the present study, ten thermo tolerant bacterial isolates belonging to genus *Bacillus* were investigated from chimneys of baking industries of Madurai, Tamilnadu, India. These isolates were given codes viz., VP1, VP2, VP3, VP4, VP5, VP7, VP8, VP9, VP10 and VP11 and colonies of these thermophilic bacteria were creamish circular on Castenholz TYE medium, cells were gram positive, rod shaped, motile and sporulating. These isolates were catalase and oxidase positive where as rest of biochemical descriptors viz., urease test, MR-VP test and fermentation of sugars were found to be negative. The optimal conditions observed for the maximum growth of all thermophilic isolates, were found to temperature of 70°C, optimal pH of 7.0 and optimal time 96 hours. The organism *Lysinibacillussphaericus* is also a sub-surface soil level species isolated from drilling mineral base oil well exploration samples in Easter planes of Columbia¹⁵. This bacterium is anaerobic, mesophilic and Gram-positive. They also dwell in soil and water¹⁵. The bacterium *Lysinibacillussphaericus* has the similarity with *Bacillus* sp. VP1 as its growth temperature varies between 40°C to 60°C and the pH range from 6 to 9. The colony is also creamy in colour with opaque edges¹⁵. *Bacillus toyonensis* is capable of producing bioflocculants which were separated from the sediment samples from the Algoa bay of South Africa. This was obtained from the clay suspension¹⁶. This organism is also having a character similar to *Bacillus* sp. VP2 as they are also having good colony formation at the temperature of 80°C. The 16S rRNA gene of all isolates was amplified, sequenced, and *insilico* analysis of these ten sequences was carried out. Based on BLAST alignment of 16S rRNA gene sequences of these isolates to GenBank sequences, these all isolates were found to belong to genus *Bacillus* and showed 98-99% similarity (Table-1).

Table I. The comparision of the 16S rRNA gene sequences of the obtained isolates with the 16S rRNA gene sequences in GenBank and their closest phylogenetic relative identity.

Isolates	Sequence		
	No.of . nucleotides	% identity	Closest phylogenetic relative (Gene bank Accession No.)
VP 1	871	98	<i>Bacillus</i> sp. vp1 (FJ848376)
VP 2	885	99	<i>Bacillus</i> sp. vp2 (FJ848377)
VP 3	900	99	<i>Bacillus</i> sp. vp3 (FJ848378)
VP 4	864	99	<i>Pseudomonassp.</i> vp4 (HM021804)
VP 5	873	99	<i>Pseudomonas</i> sp. vp5 (HM021805)
VP 7	1441	98	<i>Bacillus licheniformis</i> vp7 (HQ911359)
VP 8	1000	99	<i>Bacillus</i> sp. vp8 (JX025733)
VP 9	1000	99	<i>Bacillus</i> sp. vp9 (JX025734)
VP 10	1000	98	<i>Bacillus altitudinis</i> strain vp 10 (JX025735)
VP 11	1000	97	<i>Bacillus cereus</i> vp 11 (JX025736)

The phylogenetic analysis of the 16S rRNA gene sequences confirmed the affiliation of the thermophilic isolates with the genus *Bacillus* and *Pseudomonas* (Figure-1).

**Fig I. Phylogenetic tree showing evolutionary the relationships of *Bacillus* spp. and *Pseudomonas* spp. using between the 16S rRNA**

3.1 Gene sequences

The identification of these isolates as *Bacillus* species is in agreement with the findings of previous study⁹. It was also observed previously that the accurate taxonomic assignment depends upon the region of 16S rRNA gene that it targeted during sequencing^{10,11}. Phylogenetic research showed that thermophiles are abundant in many more extreme environments^{12,13&14}. The 16S rRNA gene, partial sequences of these ten isolates were then submitted to NCBI under these accession numbers., FJ848376 (VP1), FJ848377 (VP2), FJ848378 (VP3), HM021804 (VP4), HM021805 (VP5), HQ911359 (VP7), JX025733 (VP8), JX025734 (VP9), JX025735 (VP10) and JX025736 (VP11)..

4. CONCLUSION

In the present study, ten thermotolerant bacterial isolates belonging to genus *Bacillus*, *Pseudomonas* were investigated from chimneys of baking industries of Madurai, Tamilnadu, India. (The identification of 10 bacterial isolates belonging to the genus *Bacillus* and *Pseudomonas* based on the results of phylogenetic analysis of 10 isolates obtained). These isolates

were given codes viz., VP1, VP2, VP3, VP4, VP5, VP7, VP8, VP9, VP10 and VP11. The genomes of bacterial isolates were studies such as *Bacillus* sp. VP1, *Bacillus* sp. VP2, *Bacillus* sp. VP3, *Pseudomonas* sp. VP4, *Pseudomonas* sp. VP5, *Bacillus licheniformis* strain VP7, *Bacillus* sp. VP8, *Bacillus* sp. VP9, *Bacillus altitudinis* sp. VP10, and *Bacillus cereus* strain VP11. An attempt has been made to unearth the conserved sequences among them. Phylogenetic neighbour joining tree based on their 16S rRNA showing a relationship with related organisms has been constructed for each isolates keeping a species as an outgroup

5. AUTHORS CONTRIBUTION STATEMENT

Prof. V.Parthasarathy, carried out the research study, evaluated the results and drafted the manuscript. Dr. R. BabuRajendran, contributed to the design and implementation of the research, to the analysis of the results and reviewed the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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